

Application of a Membrane Model to the Sorptive Interactions of Humic Substances

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Humic substances, the dark-colored, natural organic polyelectrolytes that are found in practically all soils, sediments, and natural water, strongly interact with both inorganic and organic pollutants. Inorganic cationic species generally undergo complexation reactions with humic substances. The binding of cations, such as cupric ions, by humic substances often markedly reduces their toxicity to aquatic organisms. Some inorganic anionic species, in the presence of metal ions, are sorbed by humic substances. In these instances the metal ions appear to form bridges between the humic substances and the anions. Several different types of interactions take place between organic compounds and humic materials. Hydrophobic organic species partition into either insoluble or soluble humic substances. The insoluble humic substances will remove hydrophobic organic compounds from the aqueous phase, thereby rendering them less mobile. However, soluble humic substances will solubilize hydrophobic organics, increasing their mobility. Other types of interactions between humic substances and organic compounds, such as adsorption and ion exchange, also have been observed. These various interactions between humic substances and pollutants are important in governing their fate and movement in natural water systems, and, for this reason, a detailed understanding of the mechanisms of the interaction is important. A recently developed membrane model of the structure of humic substances is described; this model enables one to better understand the physical-chemical properties of these materials.

Introduction

The purpose of this paper is to present a unified picture of the interactions of humic substances with pollutants in natural systems. This paper will show how the membrane model of the structure of humic substances proposed by Wershaw (1) allows one to rationalize the various observed properties of humic substances. It is important that the reader realize, at the very outset, that the model is still under development and that modifications and refinements no doubt will be required as more data are collected. As with any model, further research may prove that it is invalid and that a new model is needed. However, at the present time this is the best model that we have available to us.

Humic substances (humic acid, fulvic acid, and humin), the dark-colored organic compounds that are present in practically all soils and sediments, have been the object of scientific inquiry for more than 200 years (2); however, the chemical structure or structures of this group of compounds is still very much a subject of debate.

In the classification of humic substances, three different fractions are operationally defined on the basis of their solubility in acid and base. These three fractions are: humic acid, fulvic acid, and humin. In the most commonly used extraction procedure, the humic and fulvic acids are extracted from soils or sediments with

sodium hydroxide. The fraction of the humic substance that is insoluble in sodium hydroxide and, therefore, remains in the soil or sediment after the extraction is called humin. The fraction of the extract that precipitates when the pH is lowered to 2 is called humic acid, and the fraction that remains in solution is called fulvic acid.

Stevenson (3) has reviewed the various chemical structures that have been proposed for humic and fulvic acids. In general, two types of structures have been proposed: highly cross-linked networks of mainly aromatic and aliphatic structural components and random aggregates of highly substituted aromatic acids and phenolic acids held together by weak interactions, such as hydrogen bonds. The latter type of structure has been proposed by Schnitzer and Khan (4) for fulvic acid; whereas cross-linked structures have been applied, generally, to humic acids. The Schnitzer and Khan structure for fulvic acid is a loose, open one that is "... punctured by voids or holes of different dimensions which can trap or fix organic molecules. ..." (4).

Wershaw (1) has shown that a much better model for humic substances is one in which the humic substances are composed of the partially degraded molecular components of living organisms, which are held together in ordered, membranelike or micellelike, aggregated structures by weak interactions, such as hydrogen bonding, pi bonding, van der Waals interactions, and hydrophobic forces. These aggregated structures con-

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stitute a separate phase in soil-water and sediment-water systems. Many of the most important physical-chemical properties of this humic membranelike phase are more a function of the structure of the membrane than of the properties of the individual molecular species of the aggregate. Evidence for this model will be outlined below; a more complete exposition of the evidence may be found in Wershaw (1) and Wershaw et al. (5).

Evidence for Model

Properties of Humic Acid

One of the first studies on the molecular aggregation of humic substances was carried out using small angle X-ray scattering. Wershaw and Pinckney (6,7) showed that soil humic acids can be fractionated by adsorption chromatography on dextran gels (Sephadex) into a series of fractions that have different chemical and physical properties. Small angle X-ray scattering measurements of solutions of these fractions showed that the molecules of the fractions form aggregates and that the degree of aggregation is a function of pH. Three different types of aggregation behavior were detected among the different fractions: *a*) some of the fractions exhibited increased aggregation below pH 3.5, with little disaggregation above pH 3.5; *b*) other fractions disaggregated up to pH 7 and then reaggregated above pH 7; and *c*) a third group of fractions exhibited a continual decrease in aggregation with increasing pH. Wershaw and Pinckney (6,7) concluded from these observations that the humic acid fractions are different chemically and that the changes in amount of aggregation of the fractions in solution most likely reflect the interaction of several different weak bonding mechanisms, including hydrogen bonding, pi bonding, and charge transfer complexation between radical species.

Examination of the fractions by C-13 nuclear magnetic resonance (NMR) spectroscopy has confirmed that the fractions are chemically different (8). The NMR spectra of these fractions have many structural features that are similar to the major chemical components of plants. Some of the fractions exhibit sharp, well-defined carbohydrate bands, as one would expect from compounds that contain carbohydrate groups, such as hydrolyzable tannins. Wershaw and Pinckney (9) reported that in some instances these fractions are humic acid-clay complexes. The complexes they isolated appear to be composed of three different types of chemical species: humic substances, proteins or amino acids, and kaolinite clay. Deamination of the clay-humic complexes with nitrous acid releases the humic substances from the clay. The authors concluded from this that the humic substances are bound to the negatively charged clay surfaces by amino acids or proteins.

Other fractions have spectral features that one might expect from ligninlike components attached to carbohydrate groups. In these fractions a strong band at approximately 180 ppm probably is due to the carbonyl resonance of carboxylic acid groups. These carboxylic

acid groups may be attached to carbohydrates forming uronic acids, or they may be attached to lignin components. Preliminary studies indicate that we should be able to distinguish aliphatic from aromatic carboxylic acids by the position of the carbonyl resonance, and therefore, it should be possible to determine the relative amounts of the aliphatic and aromatic acids in these fractions. The spectrum of one fraction is different from those of the other fractions; it appears to be mainly a phenolic oligomer, such as a tannin. These results taken together suggest that these fractions are partially degraded plant components that still retain their basic chemical structural features.

The fractionation procedure that was employed in isolating these fractions is such that one would not expect it to rupture chemical (covalent) bonds, and therefore any changes that take place during fractionation probably only involve breaking of weak bonds (e.g., hydrogen bonds, pi bonds, hydrophobic interactions). The only time when covalent bonds could be broken is during the extraction of the humic substances from soils or sediments with sodium hydroxide. Here one might expect that some weak ester linkages could be ruptured, but if oxygen is excluded from the system one would not expect any more extensive damage.

Although the fractionation on Sephadex probably is mainly a chemical fractionation, the X-ray scattering data also indicate that, in general, the fractions that elute earlier on the column are composed of larger molecules than the later eluting fractions (6,7). The earlier eluting fractions, therefore, probably have undergone less degradation. One might expect them, then, to be more homogeneous than the later eluting fractions, and the NMR spectra seem to bear this out.

Wershaw (1) concluded from the evidence outlined above that humic acids consist of a relatively homogeneous mixture of plant degradation products that aggregate together through weak interactions. Hydrogen bonding is one of the major aggregation mechanisms, as indicated by the fact that methylation of carboxylic acid and hydroxyl groups reduces the sizes of the humic acid aggregates (9,10).

As will be discussed, the fact that humic acids are surface active provides further insight into the structure of humic acid aggregates. Molecules exhibit surface activity when they possess a hydrophobic part and hydrophilic part; such molecules are called amphiphiles. Solutions of these molecules in water are not completely homogeneous because there is a higher concentration of the solute molecules at any surface where the aqueous solution comes in contact with a hydrophobic surface than in the interior of the solution. Thus, at water-air, water-mineral, and water-organic solvent surfaces there will be increased concentrations of amphiphile molecules. The hydrophobic parts of amphiphile molecules at such a surface will tend to be excluded from the aqueous phase, whereas the hydrophilic parts of the molecules will be in the aqueous phase. At amphiphile concentrations greater than the critical micelle concentration, a similar phenomenon takes place in the bulk

solution phase where the amphiphile molecules form ordered aggregates (micelles) in which the hydrophobic parts of the molecules are in the interiors of the micelles, isolated from the water molecules, and the hydrophilic parts of the molecules are on the surfaces of the micelles, in contact with the water molecules.

Humic acids are surface active, significantly lowering the surface tension of water (11–15). Both Tshapek and Wasowski (16) and Rochus and Sipos (13) have attempted to measure critical micelle concentrations (CMC) for several different humic and fulvic acids. In general, they found CMC values on the order of 1%. These studies indicate that humic acids combine into micellelike aggregates; however, as we have pointed out, the aggregation of humic substances is much more complex than a simple formation of micelles from monomeric units.

Tshapek and Wasowski (15) measured interfacial tension both at water-air interfaces and at water-benzene interfaces. They found that the addition of a given amount of humic acid caused an equal decrease in surface tension at the water-air interface as at the water-benzene interface. These results provide additional evidence that humic acid molecules are amphiphiles (that is, they have a hydrophobic part and a hydrophilic part).

Properties of Humin

Substantial evidence exists to indicate that the humin fraction of soils is also an aggregate of components held together by weak bonds. Cheshire et al. (16) reported that practically all of a soil polysaccharide can be extracted after methylation of the whole soil with dimethylsulfinyl carbanion and methyl iodide (Hakomori methylation procedure). Their results further indicated that a large portion of the polysaccharide in soil is found in humin fraction; that is to say, it is insoluble both in acids and in bases. The fact the polysaccharides can be readily released by methylation strongly suggests that they are held in the humin structure by hydrogen bonding between, for example, hydroxyl groups. Derivatization of the hydroxyl groups will disrupt the hydrogen bonding and will release the polysaccharides.

Further evidence that the humin fraction of soils and sediments is composed of components that can be separated by relatively gentle techniques is provided by the work of Allen et al. (17) and Beckwith and Nayyar (18). Allen et al. have developed a totally new approach for the extraction of essentially all of the organic substance from a soil; it includes a method for the fractionation of humin. In their method the soil or sediment first is stirred with a NaOH solution. The resulting suspension is then acidified with HCl and extracted with methylisobutyl ketone (MIBK). The humic acid and humin partition into the MIBK phase, leaving the inorganic material and most of the fulvic acid behind in the aqueous phase. A fraction similar to humic acid is then isolated from the MIBK by extraction with aqueous NaOH. Allen et al. (17) have concluded that the organic material that remains in the MIBK is the humin fraction

of soil organic matter, which normally is not extractable. They have found that this humin may be fractionated into a hydrophobic fraction and a hydrophilic fraction by extraction of the MIBK phase with water. The fraction that remains in the MIBK is white in color, and is, at least partially, soluble in hexane, chloroform, and benzene-methanol mixtures. From this they have concluded that this fraction is probably composed of lipids. The fraction that is extracted by the water phase is composed of two subfractions: an inorganic mineral phase that settles out with time, and a humic acidlike subfraction that remains in solution.

In a very interesting study (18), 8 M urea has been shown to greatly enhance the effectiveness of sodium bicarbonate, sodium orthophosphate, and sodium pyrophosphate solutions to extract humic substances from soil. Further enhancement of extraction efficiency was obtained when the soil suspensions in the extracting solvent solutions first were subjected to 5 min of ultrasonic dispersion. The most effective extraction procedure that Beckwith and Nayyar (18) found was use of the ultrasonic dispersion with a solution 0.05 M in sodium pyrophosphate and 8 M in urea. This solution was substantially more effective than 0.5 M sodium hydroxide on the four soils that they studied. Beckwith and Nayyar (18) attributed the increased extraction efficiency in the presence of urea to the ability of urea to disrupt hydrogen bonding. Urea also interferes with some hydrophobic interactions, and it commonly is used to disrupt biological membranes.

Properties of Fulvic Acid

Substantial evidence exists that indicates that fulvic acids, even though they are of relatively low molecular weight (19), are aggregate structures. In recent NMR studies in our laboratory, we have shown that fulvic acids can be fractionated into two chemically different fractions by adsorption chromatography on Sephadex G-10. Each of the two Sephadex fractions and the unfractionated fulvic acid were examined by small angle X-ray scattering. The three X-ray scattering curves are given in Figure 1. These scattering curves were measured in 1% aqueous solutions of the fulvic acid and the fractions. Small angle X-ray scattering gives a direct measure of the size of the scattering particles in solution.

The X-ray scattering data were analyzed using the method developed by Guinier (20). Guinier has shown that for an ensemble of randomly oriented identical scattering particles in which there is no long-range order, the scattering intensity, I , may be represented to a close approximation by the equation:

$$I = I_e N n^2 \exp \left[- \frac{h^2 R^2}{3} \right] \quad (1)$$

where I_e is the scattering intensity that would result if a single electron were substituted for one of the scattering particles, N is the total number of particles in the ensemble, n is the number of electrons per particle,

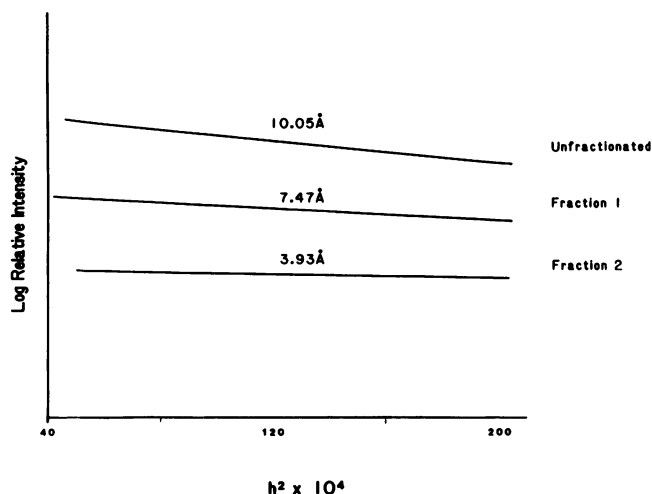


FIGURE 1. Small angle X-ray scattering curves of a 1% solution of Suwannee River fulvic acid at pH 6.

R is the radius of gyration of one of the particles, and $h = 2\pi\sin 2\theta/\lambda$, where 2θ is the scattering angle, and λ is the wavelength of the impinging X-radiation. This equation may be rewritten in the form:

$$\ln I = -\frac{h^2 R^2}{3} + \text{constant} \quad (2)$$

The radius of gyration, R , of a particle, defined as the root mean square distance of the electrons in the particle from the center of mass of the particle, is a useful parameter for comparing the sizes of different particles. Eq. (2) shows that for a system in which all of the particles in the system are of the same size that a plot of $\ln I$ versus h^2 will be a straight line and that the radius of gyration of the particles may be calculated from the slope of the line. The radius of gyration for the fractions and the unfractionated fulvic acid are shown in Figure 1. The radius of gyration of each of the two fractions is less than that of the unfractionated fulvic acid. These results show that this fulvic acid is composed of aggregates of different size particles that are held together by weak bonds that can be disrupted by adsorption chromatography. What is so surprising about the results is that two fractions and the unfractionated material are all apparently monodisperse (as indicated by the linear Guinier plots).

In natural waters the aggregation is more complex because other components are present. Hunter and Lee (21) have shown that in natural waters humic and fulvic acids form aggregates with each other and with the organics present in the waters. These aggregates have the ability to solubilize hydrophobic organic compounds.

Chen and Schnitzer (22) reported that fulvic acids are similar to humic acids in that they are also surface active. This surface activity is manifested by lowered surface tensions in fulvic acid solutions. These results indicate that at least some of the fulvic acid molecules are amphiphilic. The surface-active properties of humic sub-

stances are important in explaining their interactions with hydrophobic compounds in natural water systems, as we shall now discuss.

Sorption Interactions of Humic Substances

Information about the interiors of the humic substance aggregates has been obtained from studies on the solubilization of hydrophobic compounds in water by dissolved humic acids and from studies on the sorption of hydrophobic compounds from water solutions by soils. Wershaw et al. (5) found that a 0.5% solution of the sodium salt of humic acid increased the solubility of DDT in water by a factor of 20. More recent studies by Cary Chiou (personal communication) indicate that the factor is closer to 100. Other recent studies have shown that many different humic and fulvic acids solubilize a variety of hydrophobic compounds (23,24); therefore, it seems reasonable to assume that the solubilization of hydrophobic compounds is a general characteristic of soluble humic substances. Wershaw et al. (5) found that the presence of 0.1 N sodium chloride in the solubilization did not alter the amount of DDT solubilized. They concluded from this observation that "... the solubilization of DDT is not due to coulombic attraction of DDT to the highly charged surface of the micelles" (5). The most likely explanation for the solubilization is that the hydrophobic solute molecules partition into the hydrophobic interiors of micellelike humic aggregates. A similar process takes place in systems in which the humic substances are present as an insoluble phase. Chiou and his co-workers (25) have shown that the sorption of hydrophobic pesticides by soils from aqueous solutions is best explained as a partitioning process of the pesticides from one liquid phase (the water solution) to another liquidlike phase (the interior of the insoluble humic aggregates).

Features of Model

In light of the data outlined above, Wershaw (5) has proposed that humic acids and humins in soils and sediments consist of a number of different oligomers and simple compounds that arise from the partial degradation of plant remains. Some of these degradation products consist mainly of phenolic groups, whereas others are predominantly composed of aliphatic acids, carbohydrates, and lipids. These partial degradation products are stabilized by incorporation into the humic aggregates. The molecules are bound together by weak bonding mechanisms, such as hydrogen bonding, pi bonding, and hydrophobic interactions, into structures with hydrophobic interiors and hydrophilic exterior surfaces, thus forming structures that are similar to micelles, membranes, and vesicles (26). For simplicity in our discussion, we shall refer to the general structure of humic substances in soils and sediments as membrane-like; but, at the present time, we cannot specify the precise geometrical configurations of the structures. No doubt, in many instances, the humic substances ac-

tually form bilayer membranes around mineral grains; however, in other instances the humic substances are probably present as vesicles or micellelike aggregates. These humic membranelike structures apparently are thermodynamically stable in natural systems, but they can be partially disrupted by basic solvents and more completely disrupted by the procedures of Allen et al. (17) as we have pointed out.

The fulvic acid aggregates, because of their small size (19), may not possess distinctly separated hydrophilic and hydrophobic zones as those proposed for humic acid and humin aggregates. However, Hunter and Lee (21) have shown that fulvic acids form aggregates with humic acids in nature, and these aggregates probably consist of a hydrophobic interior with hydrophilic exterior surfaces. If fulvic acids possess substantial aromaticity, as recent NMR studies seem to indicate (27), then, in addition to the possibility of hydrogen bonding between acid and hydroxyl groups, the possibility exists for π -bonding interactions. Comparison of the molecular weights of the fulvic acids, measured by vapor pressure osmometry, and the radii of gyration of these fulvic acids suggest that fulvic acids are compact, prolate ellipsoids in solution (28).

The proposed humic membranelike aggregates are similar to biological lipoprotein membranes, in that they have hydrophobic interiors and hydrophilic exterior surfaces. The major components of most biological membranes are lipids. In general, most lipid molecules consist of a hydrophobic, hydrocarbon tail and a hydrophilic, polar (ionic) head group. In many biological membranes, the lipid molecules are arranged in two parallel rows in such a way that the hydrophobic tails of the molecules form the interior of the membrane, and the hydrophilic, polar head groups form the external surfaces. The resulting sheetlike structure is called a bilayer. Imbedded in the lipid biomembranes are less abundant components such as proteins and carbohydrates.

Membranes, however, need not be composed only of lipids; any amphiphilic molecular species can aggregate to form a membrane, and indeed, if one considers micelles and vesicles to be manifestations of membranelike behavior, then a wide variety of different membranelike systems have been observed (26). Since micelle formation and membrane formation are manifestations of the same type of phenomenon, then the proposal that humic substances form membranelike structures in soils is, to a large extent, a logical consequence of our previous finding that humic acids form mixed aggregates (micelles) in solution (7).

The hydrophobic interior of a membrane or micelle need not necessarily be composed only of nonionic hydrocarbons; it also may consist of parts of molecules that contain some polar function groups if these polar functional groups are bound together by weak interactions, such as hydrogen bonding, to form more or less hydrophobic aggregates (29). Thus, the sterol portions of bile salts can hydrogen bond to one another and then

enter into the hydrophobic interiors of bile salt-lipid micelles (30).

The molecules of many of the chemical components of plants and other living organisms are elongated, rod-like or threadlike structures that, under suitable conditions, will form ordered aggregates in water solutions (liquid crystals). These liquid crystals in which the elongated molecules are dispersed in a solvent are called lyotropic liquid crystals. In general, the compounds that form lyotropic liquid crystals in water are amphiphiles. For example, many soaps and detergents form liquid crystals in aqueous solutions. More importantly, however, many polysaccharides, proteins, and lipids that are present in plants and animals also form liquid crystals in aqueous solutions, and, in fact, biomembranes are a manifestation of this tendency (31).

The type of aggregate that is formed by an amphiphilic molecular species in solution is a function of concentration. At low concentrations (below the CMC) the amphiphile molecules will be present as monomers. At higher concentrations, the molecules will form micelles, and at still higher concentrations, the micelles will coalesce into larger micelles and ultimately into a separate liquid crystal phase (26).

In multicomponent systems in which more than one amphiphile is present, ordered aggregates also may form. An example of a multicomponent system, which has been extensively studied, is the system: lecithin, sodium cholate, and water (32). In this system, both the bile salt, sodium cholate, and the lecithin are amphiphiles, and as such, each will form micelles if present alone in solution. When the two are present together, cylindrical mixed micelles are formed with the sodium cholate molecules arranged around the peripheries of the cylinders and the lecithin molecules in the interiors of the cylinders (Fig. 2). Increasing the ratio of lecithin to bile salt causes an increase in the size of the micelles. In these micelles the polar groups of the bile salt molecules are in contact with water molecules both along the sides and the ends of the cylinders. In this regard, the mixed micelles are similar to pure bile salt micelles.

Many elongated plant molecular species that are not strictly amphiphiles, but that have polar groups more or less evenly distributed along their lengths, also form aggregates in solution. For example, polysaccharides, tannins, and other plant components often form hydrogen-bonded aggregates in solution. In case of plant polysaccharides this aggregation leads to the formation of gels such as those formed by plant pectins and gums. Rees and Welsh (33) have reviewed the formation of polysaccharide aggregates, and they discuss in detail the molecular structural features that enhance aggregation. It is well known that tannins form hydrogen bonded complexes with proteins (34); however, Benoit et al. (35) have shown that tannins also form hydrogen-bonded complexes with glucuronic acid, pectin, polygalacturonic acid, rye hemicellulose, and cellulose.

The humic substance membrane model is shown diagrammatically in Figure 3. It is composed of elongated molecules of partially decomposed plant degradation

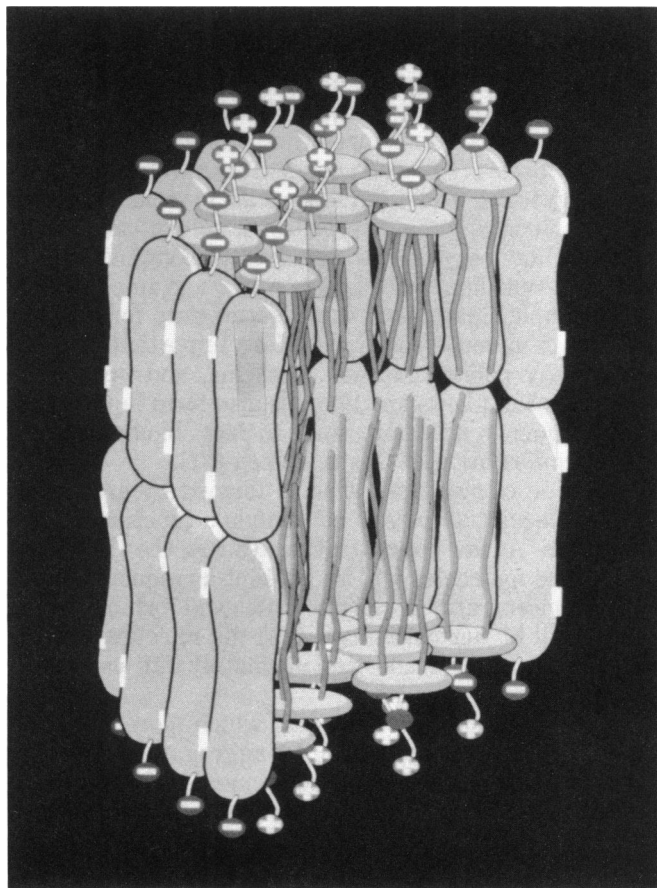


FIGURE 2. Lecithin-bile salt mixed micelle. (A) Sodium cholate molecule; (B) lecithin molecule.

products, some of which are amphiphiles and some of which are not. A generalized molecule is shown in Figure 4. In this model the membrane is pictured as having a different composition in different regions. This has been done in an attempt to diagrammatically differentiate between humic acid and humin. However, at this stage of the model, we do not know the actual spatial distribution of the constituent molecules of humic substances in soils or sediments. In this diagram, the region representing the humin fraction is pictured as being similar to a biological membrane, in that the basic membrane structure is composed of lipid molecules with the other components embedded in the lipid structure. The hydrophobic tails of the lipids form the interiors of the membrane, while the hydrophilic head groups are on exterior surfaces of the membrane. The humic acid part of the membrane is shown as being devoid of lipid molecules. In a classical lipid membrane, the interior of the membrane is uniformly hydrophobic. However, in the humic membrane it is proposed that portions of the hydrophobic interior are composed of molecules that have polar functional groups, the hydrophilicities of which are neutralized by hydrogen bonding to other polar groups. This type of structure is well known in bile salt systems (30). In addition to hydrophobic effects,

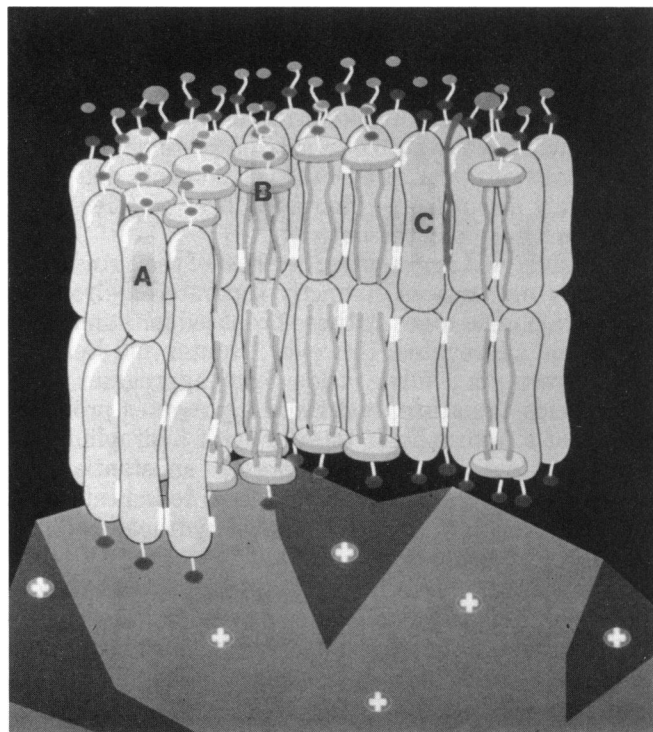


FIGURE 3. Schematic representation of humic membrandlike structure (upper part of diagram) attached to a hydrous oxide surface (lower part of diagram). The hydrous oxide surface is pictured as being positively charged. (A) Nonlipid amphiphile molecules, (B) lipid amphiphiles (pictured as a diglyceride), and (C) intercholated pollutant molecules. Both humic acid and humin components are shown in this diagram.

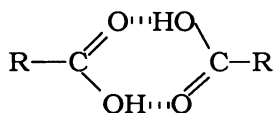
π bonding between aromatic groups is probably also important in holding humic membranes and micelles together.

Formation of Membrandlike Structures

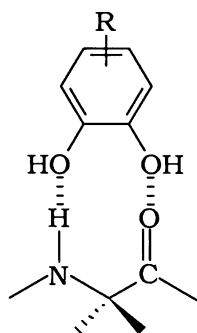
In this section, we shall discuss the question of how the humic micellelike structures form in soils and sediments. Tanford (29) has shown that lipid bilayer membranes, the existence of which are crucial to life as we know it, form spontaneously in aqueous systems when certain types of lipids are present. As Tanford (29) has demonstrated, the formation of a structure, in which the hydrophobic parts of amphiphilic molecules, such as lipids, are isolated as much as possible from the water molecules in an aqueous system results in an increase in the entropy of the system, and this increase is the driving force in the formation of structures such as micelles and membranes. As Fendler (26) has pointed out, many other amphiphilic compounds also form micelles and membrandlike structures, and these structures can be used to study interactions that take place in biological membranes.

In a manner similar to biological membranes, humic membrandlike structures will form spontaneously from elongated, rodlike or threadlike plant or animal deg-

radation products. The humic membranelike structures that form from the coalescing of these degradation products probably are less homogeneous than most biological membranes. The molecules of some of these degradation products are classical amphiphiles with well-defined hydrophobic and hydrophilic parts; however, the molecules of other species, such as uronic acids and hydrolyzable tannins, have polar groups distributed more or less uniformly along the lengths of the molecules. These nonamphiphile molecules also can be incorporated into the humic membranelike structure, if they can form aggregates in which the polarities of parts of the molecules are neutralized. Examples of this neutralization would be the hydrogen bonding of acid groups:



the complexation by the orthodihydroxyphenolic groups of tannins to the keto-imide groups of proteins (34);



and the hydrogen bonding between hydroxyl groups of carbohydrates to form double helices (36). These types of aggregates form spontaneously in solution. Thus, a two-step process is responsible for the incorporation of nonamphiphiles, such as carbohydrates, into the humic membranelike structures; in the first step the carbohydrate chains form hydrogen-bonded aggregates that reduce the polar character of parts of the molecules. The more hydrophobic segments of the aggregates then are forced into the interior of the membrane by hydrophobic interactions. The interior of the humic membranelike structure that forms from degradation products consists, therefore, not only of nonpolar molecular segments but also of aggregates of polar segments.

Interaction of Humic Substances with Inorganic Species

A relatively extensive literature exists on the interaction of metal ions with humic substances; much less work has been done on the interaction of humic substances with anionic species. Most of these studies have been concerned with the reaction of an isolated humic or fulvic acid with a single metal or anionic species. In general, it is very difficult to make a detailed comparison

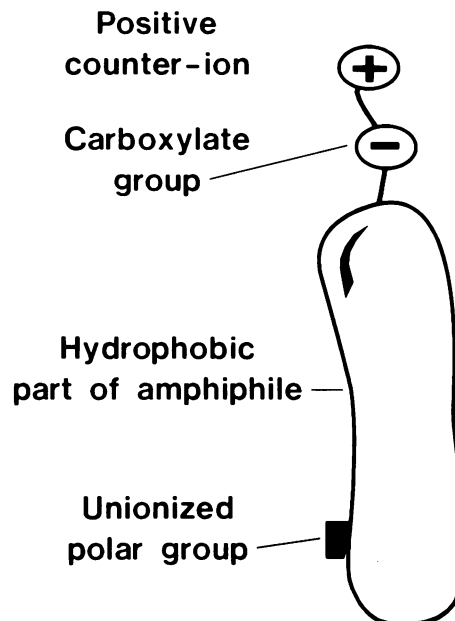


FIGURE 4. Schematic representation of a generalized, nonlipid amphiphile humic material molecule. For simplicity only one carboxylate group and one unionized polar group are shown, but in some instances more of these groups may be present on the same molecule.

of the results from one of these experiments with another because different humic and fulvic acids have been used by different workers, and because of variations in concentration and ionic strength. Therefore, we shall be concerned here only with a general overview of the subject.

Cationic Species

The complexation of metals by humic substances is of particular interest because this complexation alters the toxicity and bioavailability of metal ions in both aquatic and soil systems. Florence and Batley (37) have pointed out that strongly complexed metals, or metals attached to colloidal particles, generally, are much less toxic than free, hydrated metal ions. However, recent studies by Winner (38) and Winner and Gauss (39) indicate that, at least as far as humic acid complexes are concerned, a more complex relationship between toxicity of metal ions and the presence of a complexing anionic species exists. Winner (38) found that the addition of humic acid to water decreased the acute and chronic toxicity of Cu(II) to daphnids, but increased the acute and chronic toxicity of Cd(II) to these organisms. Winner (38) suggested that these observations may be related to the concentrations of free Cu(II) and Cd(II) present in solution, in as much as reports in the literature indicate that uncomplexed Cu(II) concentrations are reduced in the presence of dissolved humic acid and Cd(II) concentrations are not. However, Giesy (40) has found that Cd(II) ions are more strongly bound by

aquatic humic substances than Cu(II). These observed differences may be due to differences in humic substances or the experimental conditions used in the collecting of data. Such differences emphasize the care that must be exercised in attempting to draw conclusions from data collected by different workers using different humic substances and experimental conditions.

The interaction of humic substances with metals is important in plant nutrition. Jarvis (41) has pointed out that the availability of metal ions as nutrients to plants in soils is a function of the speciation of the metals in the soil solutions surrounding the plant roots. However, a detailed understanding of how humic substances alter the uptake of metals by plants is not yet available.

The movement of metals in streams, lakes, and estuaries also is a function of the types of metal complexes that are formed. Metals can exist in natural waters in a number of different forms. Florence and Batley (37) have classified these forms into the following categories:

- Particulate (metals incorporated into sediment particles)
- Simple hydrated metal ion
- Simple inorganic complex
- Simple organic complex
- Stable inorganic complex
- Adsorbed on inorganic complexes
- Adsorbed on organic complexes.

Therefore, for a given metal, a complex set of equilibria will be established between the various forms of the metal that can exist under a given set of thermodynamic conditions. If conditions such as ionic strength, salinity, pH, Eh, or sediment-load change, then a new set of equilibria, and, in general, a new distribution of the metal between the various forms, will be established.

Humic substances exist both in the sediment and in the dissolved phase. In the sediment phase the humic substances are present as coatings on mineral grains and possibly, in some instances, as separate particles. The fact that humic substances are present as thin coatings on the sediment grains causes the humic substances to be one of the most important components in a sediment, even when the organic carbon content of the sediment may be only a few percent. Thus, the distribution of metals between the dissolved and sediment phase of a water body will involve interactions with humic substances in both phases. A number of workers (42,43) have begun to attempt to unravel these interactions, but, so far, progress has been very slow.

The most common type of interaction of metal ions with humic substances is ion exchange, in which protons on carboxylic acid groups are replaced by metal ions. Marinsky and his co-workers have developed a physical-chemical description of protonation and metal ion complexation by humic and fulvic acids (44-46). This description is based on a model developed by Marinsky (47) for weakly acidic polymeric gels. Marinsky (47) showed that there are two types of gels: those that are permeable to low molecular weight salts, and those that are impermeable to low molecular weight salts. Mar-

insky (47) concluded from his observations that polymeric gel molecules, even when completely dissolved in water, must be treated as a separate phase from the bulk solution. This conclusion provides a powerful new way to rationalize the results of acid-base titration studies of humic substances, which, as has been shown above, exist as separate membranelike or micellelike phases in natural water systems.

In gel molecules that are permeable to salts, a Donnan potential term must be included in the calculation of the intrinsic dissociation constant of the repeating acidic exchange units. The permeable molecules behave like a separate phase that is separated from the bulk solution by a semipermeable membrane. In reality, the fact that the exchange sites in the polyelectrolyte phase are fixed provides the nondiffusible component that is required for the existence of a Donnan potential.

If the polyelectrolyte molecules are not permeable to low molecular weight salts, a Donnan potential is not set up. However, the effect of surface charge must be taken into account. Marinsky (47) has shown that, although the effective concentration of counter ions (salt cations and protons) at the surface of a negatively charged polyion is different than in the bulk solution (48), the ratio of the activities of protons to salt cations at the surface is equal to the ratio of activities protons to salt cations in the bulk solution. Marinsky's (47) analysis allows one to calculate, from acid-base titrations of the polyelectrolyte gel at different background electrolyte concentrations, the intrinsic ionization constants of the repeating units of a weakly acidic polyelectrolyte. One may further distinguish from the titration data between a permeable and a nonpermeable gel.

Application of Marinsky's (47) analysis to fulvic acid titration data (45) indicated that fulvic acid molecules, in general, behave as rigid polyions that are impermeable to salt ions. Preliminary work indicates that humic acids in solution behave in a similar fashion, as one would expect if they exist as micellelike structures with all of the charged groups on the exterior surfaces of the micelles. The exchange sites of the fulvic acids molecules, however, are not uniform, as was the case for the polymeric gels used by Marinsky (47).

Several different approaches have been used to account for the apparent nonuniformity of the complexing ligands in humic and fulvic acids. Fish et al. (49) have reviewed the various mathematical models that have been applied to humic and fulvic acid titration data. In general, two categories of models have been used: discrete-ligand models and continuous-multiligand models. In the discrete-ligand models one assumes that the measured titration data result from complexation by only a small number (generally two or three) of different types of ligands. In the continuous-multiligand models it is assumed that a very large number of different types of binding sites are present in humic and fulvic acids, which can be represented by some sort of continuous distribution function. Fish et al. (49) have shown that the continuous-multiligand models have no advantage over the discrete ligand models, and they are more dif-

difficult to apply to experimental data. Fish et al. (49), therefore, concluded that the discrete-ligand models generally are preferable. Ephraim et al. (45) have shown that the titration data for a typical fulvic acid can be represented by two different types of ligands.

The simple picture of metal ions interacting with discrete humic and fulvic acid polyions or micelles in solution is complicated by the fact that polyvalent cations cause aggregation of humic and fulvic acids (50–52). This aggregation probably will require the inclusion of a Donnan potential term into the equations that represent the metal complexation.

Humic and fulvic acids also form charge-transfer complexes with metal ions. Most of the evidence for these complexes exists for Cu(II) (51,53,54). The electron donating groups in these complexes appear to be carboxylate groups (55). However, some evidence also exists for coordination to N-containing groups (56). Piccolo and Stevenson (57) and Senesi et al. (58) have also found evidence from infrared studies that, in addition to carboxylate groups, carbonyl groups participate in the binding of some metal ions to soil humic substances. Senesi et al. (58) have found that soil humic acids have two different classes of binding sites for Cu(II) and Fe(III); stronger sites in which the metal-ligand complexes are covalent in character and which are stable to proton exchange, and sites that form weaker complexes that are readily disrupted by protons; VO^{2+} ions are also bound by the weaker sites, but are readily displaced by Cu(II) and Fe(III).

Humic substances are active intermediaries in the photochemical reduction of Fe(III) in natural waters (59). Apparently the reduction takes place through the formation of charge-transfer complexes between Fe(III) and the carboxylate groups of the humic substances. The reduction reaction takes place in the dark, but is accelerated in the presence of light. The ligand-to-metal charge transfer results in the formation of CO_2 and other oxidation products from the humic molecules (59).

Insufficient data exist for a detailed understanding of the coordination of metal ions to humic substances. A few studies have used electron spin resonance (ESR) to determine the orientation of humic substance ligand around Cu(II) and Fe(III) ions (53,55). However, much more data will be required before a general picture emerges. It does appear that ligands from more than one humic or fulvic particle can be complexed by a single metal ion, thus bringing about aggregation of these humic or fulvic acids (50,52).

In an interesting study, Jouary and Chassin (60) found that humic acid aggregates precipitated by excess iron or protons were hydrophobic, whereas those precipitated by calcium ions were hydrophilic. In the case of iron the most likely explanation is that the iron ions bridge between the polar groups such that nonpolar parts of the molecules that make up the humic acid aggregates are on the exterior surfaces of the aggregates. In the case of calcium however, apparently, the metal ions only neutralize the surface charge, but do

not bridge between particles, so that the nonpolar groups are not exposed on the exterior surfaces of the aggregates. In the case of protons, the neutralized carboxyl groups must hydrogen bond one with another and thereby reduce the surface hydrophilicity.

Anionic Species

Most of the studies on the interaction of humic substances with anions have been concerned with the interactions of phosphorus species with humic substances. This has come about because phosphorus is an essential plant nutrient, and large amounts of it are being added to natural waters as orthophosphate in sewage. Excessive amounts of phosphorus lead to eutrophication of natural water bodies.

In the pH range of most natural waters the predominant orthophosphate species in solution is H_2PO_4^- (61). Orthophosphate reacts with Fe(III) and dissolved aquatic humic substances to form high molecular weight complexes (62). This Fe(III)-humic substance-orthophosphate complex is light sensitive; irradiation of the complex reduces the Fe(III) to Fe(II) and releases the orthophosphate (63).

At the present time, mechanisms of phosphorus cycling in natural water bodies has not yet been clearly elucidated; however, it is apparent that Fe(III)-humic substances-orthophosphate complexes are an important part of this cycling. Francko has summarized our present knowledge as follows (62):

In summary, it appears that epilimnetic P cycling, as a summation of the uptake, sequestering, and release of poorly characterized compounds, and involving biotic and abiotic processes which appear to be modulated by organic and inorganic constituents of lake water, is not readily characterized by any single process. Rather, the data presented in this paper and those published by a large number of investigators may best be resolved by postulating that P recycling represents a continuum of processes, involving abiotic complexation and release, and a number of biotic processes involving enzymatic hydrolysis, each of which is highly plastic in the natural environment.

In addition to reducing metal cations (e.g., Fe^{3+}), humic substances also reduce anions. Goodgame et al. (64) have shown that humic acid reduces Cr(VI) in the oxyanions CrO_4^{2-} , HCrO_4^- , H_2CrO_4 and $\text{Cr}_2\text{O}_7^{2-}$ to Cr(V) in the pH range between 4 and 9. At lower pH values Cr(III) is produced. In as much as Cr(VI) is carcinogenic, this is a particularly important finding. Another example of this reduction was demonstrated by An and Langquiu (65), who found that Se(VI) is slowly reduced in acidic solutions to Se(IV) by humic acid.

Interaction of Humic Substances with Organic Species

General Comments

Organic compounds interact with humic substances in a number of different ways. Nonionic organic compounds partition into insoluble humic substances in sed-

iments and soils, whereas soluble humic substances solubilize nonionic organics (11,25). Ionic organic compounds can undergo ionic exchange reactions and charge-transfer complexation. In addition, some evidence exists for oxidative coupling reactions between anthropomorphic organic compounds and humic substances. Each one of these types of reactions will be discussed. The emphasis in this discussion will be on the elucidation, as far as possible, of the mechanisms of the reactions, rather than on cataloging the various studies in the literature.

Partition Reactions

Chiou and his co-workers (25) have shown that the sorption of nonionic organic compounds by wet soils involves partitioning of the organic compounds between the soil-water phase and the soil-organic phase. Chiou et al. (25) point out that this partitioning is analogous to partitioning between water and an immiscible organic solvent phase. They have shown that the same equations hold for solvent-water partitioning and soil-water partitioning. The model presented by Chiou et al. (25) is an extension of the theory developed by Flory (66) for solutions of high molecular weight polymers in low molecular weight solvents. Flory (66) assumed that a polymer molecule is a long chain composed of segments that are about the same size as the solvent molecules. He also assumed that these segments exist more or less independently of one another as kinetic units. Thus, implicit in Chiou's work is the assumption that at least part of the humic substances in soils behave as a hydrophobic liquidlike phase, in which the hydrophobic segments of the molecules that make up the phase are free to move more or less independently of each other; this concept fits in very well with the membrane model presented here.

An analogous process to partitioning into an insoluble organic phase is solubilization of hydrophobic organic compounds by dissolved humic substances. Wershaw et al. (11) first studied this process. They found that the soluble sodium salt of humic acid enhances the solubility of DDT in water. This increase in solubility apparently is brought about by the partitioning of the DDT molecules in the hydrophobic interiors of humic acid micelles. A number of other workers have extended this work to include a variety of other hydrophobic organic compounds and other humic substances, and generally have found similar solubilization behavior (24,67).

Perdue (68) has shown that partition of an organic substrate into a humic micellelike phase can modify the rate of hydrolysis of the substrate. Acid-catalyzed hydrolysis reactions will be accelerated and base-catalyzed reactions will be inhibited. In addition to the effects on acid-base hydrolysis, partition into a humic micelle phase can also alter the general reactivity of a substrate.

Adsorption

Chiou and Shoup (69) have shown that in dry soil hydrophobic organic compounds adsorb to the surface

of soil particles. This adsorption is characterized by different equations than the partition reactions that take place in wet soils. It is not clear what the constitution of the binding surfaces is. Chiou and Shoup (69) have concluded that the adsorption takes place on mineral surfaces. However, a number of studies have shown that metal oxide surfaces in natural waters bind humic substances and that this alters the surface properties of the metal oxides (70). Clay minerals also form complexes with humic substances (9). These complexes consist of humic substances coating the surfaces of the clay particles and, in some instances, entering into interlayer positions in the clay particles (9). These results suggest that a substantial part of the mineral surfaces in soils and sediments are coated with humic substances; however, uncoated surfaces also may be present.

Ionic Interactions

A number of different types of ionic interactions have been reported between humic substances and organic compounds. In the simplest case, the organic compound exists in solution as cations that can be bound by carboxylate groups of humic substances. Amino acids and triazine herbicides would bind to humic substances by this mechanism at low pH values where the nitrogen-containing groups would be protonated (71). Senesi and Testini (71), in their study of binding of herbicides by humic substances, point out that hydrogen bonding can also take place between humic substances and basic herbicides such as substituted urea herbicides. The groups most likely to enter into hydrogen-bonding interactions would be hydroxyl and carboxylic acid groups on the surfaces of the humic substance membranelike aggregates.

Enzymes form complexes with humic substances and metal ions. In most of the studies that have been reported (72,73), the presence of metal ions was necessary for binding. In none of the cases mentioned in the review by Burns (72) has the mechanism of binding been completely elucidated. Several mechanisms have been suggested, including bridging by polyvalent cations between positively charged groups of humic substances and positive groups of the enzymes and occlusion of the enzymes in flocs formed when metals are added to humic substances in solution.

Oxidative Coupling Reactions

Humic substances contain significant quantities of stable free radicals (74). The presence of these radicals has led some workers to conclude that oxidative coupling reactions are important both in the formation of humic substances and in their interactions with other organic compounds (75). Most of the work on the oxidative coupling in humic substances has been concerned with coupling of phenolate free radicals. Evidence for the presence of radicals of this type in humic substances is obtained from electron spin resonance (ESR) studies. Although the ESR data are not completely definitive,

the presence of semiquinone radicals appears to be the best interpretation of the data.

Sarkar and Bollag (76) have shown that 2,4-dichlorophenol (2,4-DCP), a breakdown product of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), undergoes enzymatically mediated oxidative coupling reactions with humic substances. Other phenolic compounds apparently also undergo this type of reaction with humic substances (75). Nonenzymatic oxidative coupling reactions to humic substances may also take place, especially to dissolved humic substances in streams. Thus, Kotzias et al. (77) have shown that stable free radicals are produced by ultraviolet irradiation of humic substances. These free radicals then could react with other organic compounds to form coupled products.

Photochemical Reactions

Other types of photochemically induced reactions have been detected in solutions containing humic substances. Zafiriou et al. (78) have reviewed photochemical reactions in natural waters. They have shown that a number of different types of light-mediated reactions can take place in natural waters; some of these reactions involve humic substances and some do not. Zepp et al. (79) have shown that humic substances can transfer energy from absorbed sunlight to certain organic pollutants. They found that the photo-excited triplet states of the humic substances caused degradation of 1,3-pentadiene and 2,5-dimethylfuran in water. The reaction mechanism appears to involve formation of singlet oxygen, which can rapidly oxidize furans, sulfides, and electron-rich olefins.

More recently, Zepp et al. (80) reported that dissolved organic matter (including humic substances) from natural waters and soils can produce hydrated electrons when irradiated with sunlight. These hydrated electrons can be important in the photoreduction of organic pollutants.

Effect of Humic Substances on the Fate and Transport of Pollutants

As we have outlined above, humic substances interact in a variety of different ways with all the other components in natural water systems. These interactions affect the fate and transport of pollutants in these systems. Many of these interactions can be rationalized using the membrane or micelle model. However, in order to extend this model to allow us to make useful predictions will require it to be incorporated with a generalized transport model such as one of the multimedia transport models.

Cohen (81) has reviewed the status of multimedia modeling in natural water systems. He has described how these models are composed of compartments and various levels of subcompartments. Pollutants can move between compartments and subcompartments and can undergo changes in the compartments and subcompartments. All of these interactions can be represented

by differential equations. However, in order to apply the multimedia modeling techniques to natural water systems requires a detailed description of the various compartments and subcompartments.

Conclusions

Humic substances are present in soils, sediments, and natural waters in both insoluble and soluble forms. In both forms, humic substances strongly interact with organic and inorganic pollutants. The membrane model for humic substances allows one to understand how and why these interactions take place, and it provides a starting point for the modeling of physical-chemical reactions in natural water systems.

The use of tradenames in this report is for identification purposes only and does not constitute endorsement of the U.S. Geological Survey.

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